International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

Analysis Of Blashv Gene Sequencing In UTI-Associated Klebsiella Pneumoniae Isolates

[1]**Riham Adday Salman,** [2]**Kais Kassim Ghaima*,** [3]**Zinah Faeq Ahmed**
[1]Minitry of Health
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.
[3]Scientific Research Commission, Research and Technology Center of Environment, Water and Renewable Energy.
Corresponding author: *kaiskassim@gmail.com

ABSTRACT
Particularly in urinary tract infections (UTIs), Klebsiella pneumoniae, which produces extended-spectrum beta-lactamases (ESBLs), is become more resistant to various cephalosporin generations. Finding differences in the blaSHV genes of the local isolates of uropathogenic K. pneumoniae was the goal of this investigation. The PCR results for 20 specific blaSHV genes of the isolates that produce ESPL and are resistant to all beta-lactam antibiotics utilized except Meropenem and Imipenem were selected for sequencing study. The blaSHV genes were correctly identified by PCR when the acquired sequences (19) were aligned with the reference strains in GenBank. Additionally, these sequences were examined for the presence of gene variations and nucleotide differences with the reference sequence of SHV-1. The results also indicated that some isolates had the variant SHV-11. The results of detection of amino acid alterations in the products of blaSHV genes revealed that the most amino acid substitution was Arginine to Lysine (R⟶K) at the positions 70, 132 and 216. Another amino acid substitution was Ileucine to Methionine (I⟶M) at the position 24 in the isolates (RK12, RK13, RK15, and RK16). Also, changing at the position 71 by presence Proline instead of Arginine (R⟶P) in the isolates (RK8, RK9, RK10, RK13 and RK15). It was obvious that the isolate RK16 had 5 amino acid substitutions; three of them were Arginine to Lysine. The results confirmed that the most amino acids substitutions were detected in new positions not recorded in the previous studies.
Key words: *K. pneumoniae, blaSHV gene, UTI, Sequencing.*

INTRODUCTION

Nearly 50% of people will get a urinary tract infection (UTI) at some point in their lives, making it one of the most common hospital-acquired illnesses with a high mortality risk [1]. UTIs are primarily caused by gram-negative organisms, particularly those belonging to the Enterobacteriaceae family, which includes *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli* [2]. The mechanisms of acquired and inherent resistance to numerous antibiotic families are present in *K. pneumoniae*. The emergence of Extended-Spectrum Beta-Lactamases (ESBL) enzymes in *K. pneumoniae* strains has led to an increase in the prevalence of multidrug resistance [3]. Concern over multidrug-resistant Gram-negative bacteria that manufacture ESBL enzymes is developing, and hospital-acquired urinary tract infections caused by multiple drug-resistant *K. pneumoniae* have grown dramatically [4, 5]. The Enterobacteriaceae family, specifically *K. pneumoniae*, *K. oxytoca*, and *E. coli*, is the primary producer of penicillins, monobactams, and oxyimino-cephalosporins, which are hydrolyzed by ESBLs, which are frequently plasmid-mediated enzymes with multiple genotypes, including SHV, TEM, and CTX-M types [6]. Local urinary tract pathogenic bacteria produce ESBL enzymes, which have been shown in several local investigations to play a role in multidrug resistance, namely to the most prevalent cephalosporins and penicillins [7, 8]. In addition to surveying the presence of ESBL genes (*blaSHV, blaTEM*, and *blaCTX-M*) in *K. pneumoniae* isolates and assessing their prevalence among ESBL-producing local isolates, the current study aims to explore the distribution of Extended-Spectrum Beta-Lactamases (ESBLs) among *K. pneumoniae* strains isolated from patients with UTIs.

MATERIALS AND METHODS

To perform the nucleotide sequencing of the blaSHV genes, twenty isolates that were resistant to multiple drugs were chosen. Before being used, 20 µl of each gene's PCR product was kept at -20°C. Macrogen DNA Sequencing (Seoul, Korea) subsequently performed the sequencing. The ClustalW technique of the MEGA6 program was used to download and align the nucleotide sequences of the blaSHV genes of K. pneumoniae reference strains that have been reported from various locations across the world and are accessible in the public database Gen Bank. The appropriate software (MEGA6 and BioEdit) was used to translate nucleotides to amino acids and find changes in the gene sequence.

RESULTS AND DISCUSSION

According to the results of our earlier investigation, 66 (85.7%) of the 77 K. pneumoniae isolates examined for the production of ESBL by uropathogenic strains using the ESBL E-test were shown to be capable of producing ESBL enzymes. Clinical isolates showed high levels of resistance to Ampicillin, Cephalexin, Piperacillin Ceftazidime, and Amoxicillin-clavulanic acid, according to the antibiotic susceptibility test for the isolates under study. The PCR results showed that *blaSHV* was the most common gene and that 56% of the isolates had the two genes (*blaSHV* + *blaTEM*) as the predominant genotype [9].

Agarose gel electrophoresis was used to identify PCR products for 20 specific *blaSHV* genes of the isolates that produce ESPL and are resistant to all beta-lactam antibiotics, with the exception of imipenem and Meropenem. The Applied Biosystem (AB) capillary system (Macrogen Research, Seoul, Korea) was used for the sequencing. Direct sequencing was applied to the PCR products, and an automated sequencer was used to sequence both strands. The Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) was used to analyze DNA sequences and perform similarity searches.

The NCBI/GenBank database now has the nucleotide sequences of the blaSHV genes of our local isolates (RK8, RK9, RK10, RK13, RK15, and RK16) described in this investigation, with accession numbers as indicated in Table (1).

Table 1. The accession numbers for the *blaSHV* gene nucleotide sequences of local isolates of *K. pneumoniae*.

Isolate code	*bla*SHV
RK8	LC093477
RK9	LC093478
RK10	LC093479
RK13	LC093480
RK15	LC093481
RK16	LC093481

The accurate identification of *blaSHV* genes by PCR was validated by aligning the acquired sequences (19) with the reference strains in Gen Bank. Additionally, these sequences were examined to find variations in the nucleotides (mutations) and to determine whether these genes had any variants. There were some differences in the nucleotides of our queries in the various positions, including substitutions and deletions of the subject, according to the results of aligning the *blaSHV* sequences of the local isolates with the reference strain 32 isolated from UTI with accession number KP853086 (*blaSHV-1*). We can also conclude that these local isolates have novel SHV variants because it was discovered that the majority of sequences had high similarity (99%) but also contained new mutations at positions not noted in earlier studies. These variations caused changes in several amino acids of the protein (beta-lactamase

enzyme) that was produced. For instance, seven purine nucleotide changes (G to A) were found in various locations across the sequence when the *blaSHV* gene from the local isolate RK4 was aligned with the reference gene *blaSHV-1* (strain ZUKP135) (Figure 1).

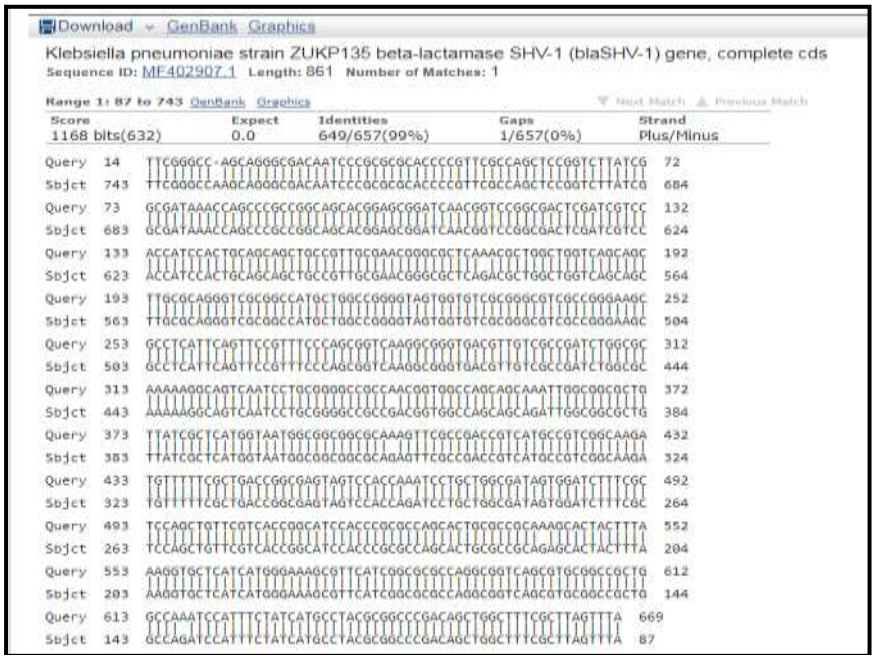

Figure 1. *Klebsiella pnumoniae blaSHV* gene sequence alignment between the reference strain (ZUKP135) in GenBank and the local isolate RK4.

It was found that the variant of *bla*SHV in the local isolates RK17 and RK19 exhibited 99% of similarity to the variant *bla*SHV-85 for *K. pneumoniae* isolated from neonatal intensive care units in Brazil (with accession number NG_050119) as showed in figure (2)

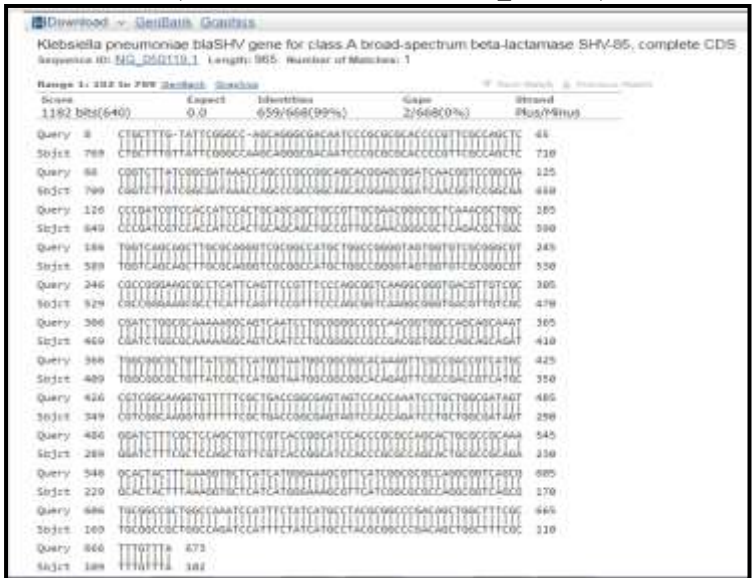

Figure 2. Alignment of *K. pnumoniae bla*SHV gene sequence of the local isolate RK19 with SHV-85 of reference strain (accession number NG_050119) available in GenBank.

The study of Tawfik *et al.*, [10] indicated to the presence of the variants; SHV-1, SHV-11 and SHV-85 in the clinical isolates of *K. pneumoniae* in Saudi Arabia, in addition to TEM and CTX-M genes.

In order to detection the results of mutations in the nucleotides sequences of *bla*SHV genes as amino acids changes, these sequences translated to the amino acids sequences by using the suitable software as showed and explained in the figure (3) and table (2) .

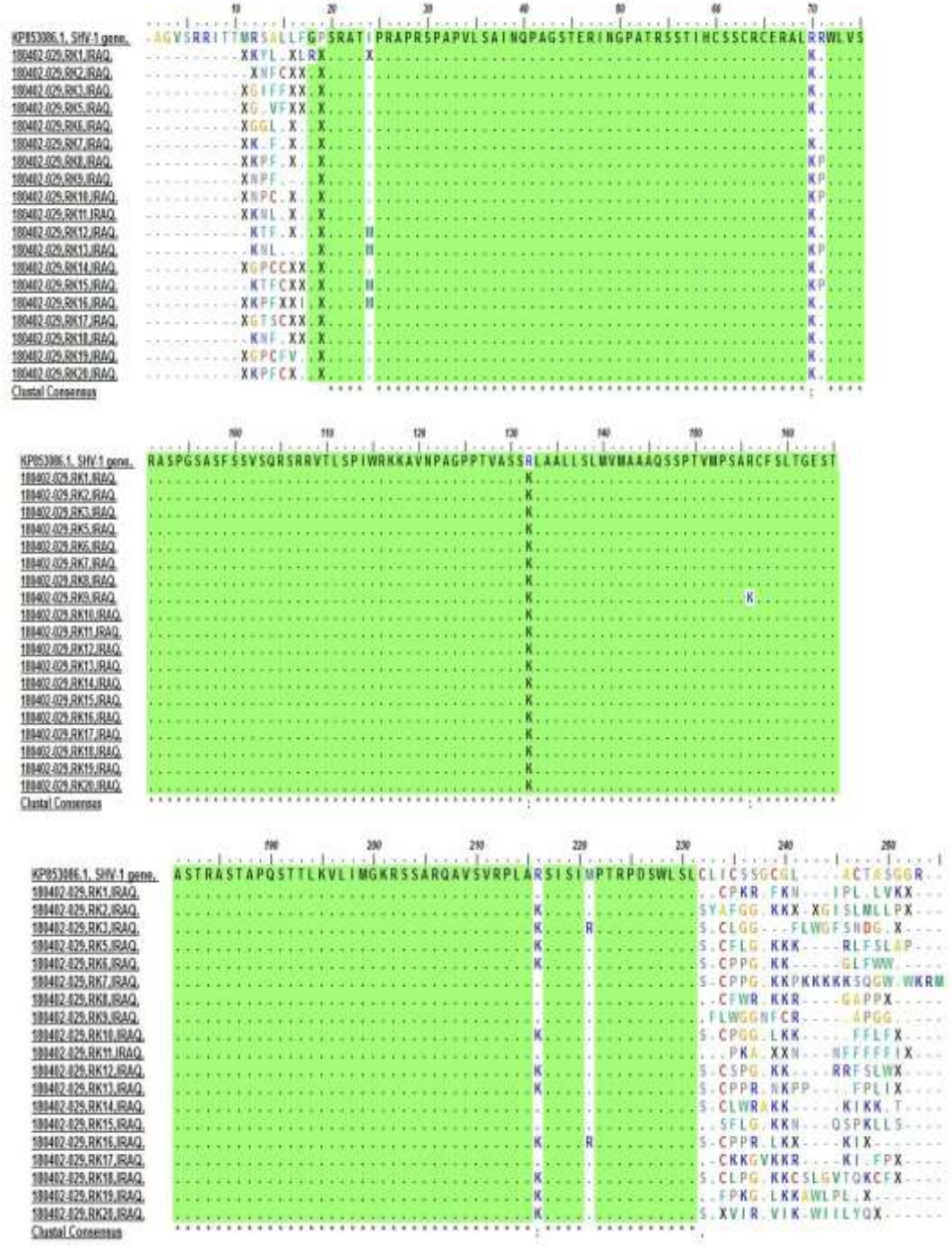

Figure 3. Alteration of amino acids in *bla*SHV detected in *K. pneumoniae* local isolate by MEGA6 and BioEdit softwares.

Table 2. Alterations of amino acids in *bla*SHV detected in the *K. pneumoniae* local isolates.

Code No.	***bla*SHV positions**

of isolates								
RK1	Gly18Arg		Arg70Lys		Arg132Lys			
RK2			Arg70Lys		Arg132Lys		Arg216Lys	
RK3			Arg70Lys		Arg132Lys		Arg216Lys	Met221Arg
RK5			Arg70Lys		Arg132Lys		Arg216Lys	
RK6					Arg132Lys		Arg216Lys	
RK7			Arg70Lys		Arg132Lys			
RK8			Arg70Lys	Arg71Pro	Arg132Lys			
RK9			Arg70Lys	Arg71Pro	Arg132Lys	Arg156Lys		
RK10			Arg70Lys	Arg71Pro	Arg132Lys		Arg216Lys	
RK11			Arg70Lys		Arg132Lys			
RK12		Ile24Met	Arg70Lys		Arg132Lys		Arg216Lys	
RK13		Ile24Met	Arg70Lys	Arg71Pro	Arg132Lys		Arg216Lys	
RK14			Arg70Lys		Arg132Lys			
RK15		Ile24Met	Arg70Lys	Arg71Pro	Arg132Lys			
RK16		Ile24Met	Arg70Lys		Arg132Lys		Arg216Lys	Met221Arg
RK17			Arg70Lys		Arg132Lys			
RK18			Arg70Lys		Arg132Lys		Arg216Lys	
RK19			Arg70Lys		Arg132Lys		Arg216Lys	

The results of detection amino acids alterations in the products of *bla*SHV genes revealed that the most amino acid substitution was Arginine to Lysine (R→K) at the positions 70, 132 and 216. Another amino acid substitution was Ileucine to Methionine (I→M) at the position 24 in the isolates (RK12, RK13, RK15 and RK16). Also, changing at the position 71 by presence Proline instead of Arginine (R→P) in the isolates (RK8, RK9, RK10, RK13 and RK15).It was obvious that the isolate RK16 had 5 amino acid substitutions; three of them were Arginine to Lysine (Table 2). The isolates with multible mutations of *bla*SHV genes were resistant to beta-lactam antibiotics such as; Cephalexin, Ceftazidime, Cefepime, Amoxicillin-clavulanic acid and

Piperacillin, excepted carbapenems. Most of the positions of mutations in the *bla*SHV gene of the local isolates were new mutations not recorded in the previous studies; maybe that's why they resist beta-lactam antibiotics with high percentages. The increasing of the spectrum activity of ESBL enzymes, such as in TEM1, and SHV1 may be due to the point mutations around the active sites of the TEM and SHV sequences have led to amino acid changes [11]. It was found that the clinical isolates possess more than one or two mutations in TEM and SHV ESBLs [12]. The study of Salah *et al*., [13] in Assiut, Egypt detected multiple mutations among beta-lactamases of *K. pnemoniae* isolated from UTI; in SHV-1, SHV-11, SHV-111 and SHV-115. The substitutions demonstrated in SHV ESBLs include mutations at positions in, or very close to, the oxyanion pocket that contains the enzyme's active site such as Ala146, Gly156, Leu169, Asp179, Arg205, Gly238 and Glu240 [12].

In their study of *Klebsiella pneumoniae* strains obtained from cefotaxime-resistant Sudanese patients, Altayb *et al.* [14] found that the substitution of arginine to lysine at position 269 of the *blaSHV* sequence resulted in a difference in protein size. This mutation will create an empty space in the protein's core, increasing resistance to these beta-lactam molecules. The study that conducted on class C beta-lactamase from C*itrobacter freundii*, it was observed that the presence of Arginine instead of Lysine in the active site results in a lowering of the affinity toward Cephalothin, Cephaloridine, and Benzylpenicillin [15]. The efficient hydrolysis of Ceftazidime by ESBL enzyme is due to serine residue at position 238, while the efficient hydrolysis of Cefotaxime is correlated with the lysine residue at position 240 [16]. Several f local studies indicted to the increasing of resistance among *K. pneumoniae* local isolates and finding the new alternative therapeutic agents to overcome the high resistance (17, 18).

CONCLUSION

The present study showed that most of the positions of mutations in the *bla*SHV gene of the local isolates were not recorded in the previous studies, and the emergence of high level of resistance to the new generations of cephalosporins in the local isolates may be due to these mutations.

REFERENCES

[1] Foxman B. 2010. The epidemiology of urinary tract infection. Nature Review Urology. 7:653–660.
[2] Nicolle L. 2013. Urinary tract infection. Critical Care Clinic. 29: 699-715.
[3] Marra A, Pereira C, Gales A, Menezes L, Cal R, de Souza J. *et al.* 2006. Bloodstream infections with metallo-beta-lactamase producing *Pseudomonas aeruginosa*: epidemiology, microbiology, and clinical outcomes. Antimicrobial Agents Chemotherapy. 50(1):388-90.
[4] Coque T, Baquero F, Canton R. 2008. Increasing prevalence of ESBL producing Enterobacteriaceae in Europe. Euro Surveillance. 13(47): 19044.
[5] Yasin SA, AL-Azawi AH. 2019. Antibacterial activity of *Conocarpus erectus* leaves extracts on some microorganisms isolated from patients with burn infection. Plant Archives. 19 (2): 583-589.
[6] Paterson D, Bonomo R. 2005. Extended-spectrum β-lactamases: a clinical update. Clinical Microbiology Reviews. 18(4): 657-686.
[7] Al-janaby A, Alhasnawi H. 2017. Phenotypic and Molecular Characterization of Multidrug Resistant *Klebsiella pneumoniae* Isolated from Different Clinical Sources in Al-Najaf Province-Iraq. Pakistan Journal of Biological Sciences. 20(5):217-232.
[8] Khalid H, Yousif S, Jubrael J. 2017. Bacteriological and Molecular characterization of extended spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* isolated from Kurdistan region, Iraq. Science Journal of University of Zakho. 1(1): 158-163.
[9] Salman RA, Ghaima KK. 2018. Prevalence of ESBL genes in ESBL producing *Klebsiella pneumoniae* isolated from patients with urinary tract infections in Baghdad, Iraq. BIOSCIENCE RESEARCH. 15(3): 2049-2059.
[10] Tawfik A, Alswailem A, Shibl A, Al-Agamy M. 2011. Prevalence and Genetic Characteristics of TEM, SHV, and CTX-M in Clinical *Klebsiella pneumoniae*Isolates from Saudi Arabia. Microbial Drug Resistance. 17(3): 30.
[11] Bradford P. 2001. Extended spectrum beta-lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. Clinical Microbiology Reviews. 14: 933–951.
[12] Gniadkowski M. 2008. Evolution of extended-spectrum beta-lactamases by mutation. Clinical Microbiology Infection. 14 (1):11-32.

[13] Salah M, Azab M, Halaby H, Hanora A. 2016. Mutations in β-lactamases detected in multidrug resistant gram negative bacteria isolated from community acquired urinary tract infections in Assiut, Egypt. African Journal of Microbiology Research. 10(46):1938-1943.
[14] Altayb H, El Amin N, Mukhtar M, Mohamed A, Hassan M, Siddig M. 2015. Detection of novel SHV Extended-Spectrum Beta-Lactamase gene mutation in *Klebsiella pneumoniae* isolated from patient in Eastern Sudan. Current Research in Microbiology and Biotechnology. 3(1): 542-549.
[15] Tsukamoto K, Tachibana K, Yamazaki N, Ishii Y, Ujiie K, Nishida N. et al. 1990. Role of lysine-67 in the active site of class C beta-lactamase from *Citrobacter freundii* GN346. European Journal Biochemistry. 188(1):15–22.
[16] Huletsky A, Knox J, Levesque R. 1993. Role of Ser-238 and Lys-240 in the hydrolysis of 3rd-generation cephalosporins by SHV-type beta-lactamases probed by site-directed mutagenesis and 3-dimensional modeling. Journal of Biological Chemistry. 268: 3690–3697.
[17] Khalid, T.W.B.M., Ghaima, K.K. 2023. Effect of Berberine as efflux pump inhibitor in multidrug-resistant Klebsiella pneumoniae isolated from urinary tract infections. Bionatura, 8(2), 73.
[18] Hamad, S.T., Ghaima, K.K. 2023. Study the Gene Expression of *bla-IMP* and *bla-VIM* Genes In Meropenem-resistant *Klebsiella pneumoniae* Isolated from Urinary Tract Infections. Bionatura, 8(CSS1), 82.